Physiological Changes, Fibroblast Cells

Fibroblasts have been used since the 1960s as a cell culture model for organismal aging. Fibroblast cultures have provided a wealth of information about basic cellular processes, such as cell cycle control, and insights into neoplastic transformation and cellular stress responses, both of which are prominent in aged organisms. The apparent aging that cells undergo in culture is a complex process, some features of which are apparent in aging organisms. Nonetheless, it is not yet clear to what extent the aging of cells in culture reflects aging in multicellular organisms.

Fibroblasts in vivo.

Fibroblasts produce and maintain the connective elements, or stroma, of most tissues. The stroma provides structure and regulatory signals to the functional cells of tissues (the parenchyma). Fibroblasts secrete extracellular matrix components of the stroma -- for example, collagens and fibronectin. In response to a wound or need for tissue remodeling, they can also secrete enzymes that degrade the extracellular matrix. In addition, fibroblasts secrete a variety of factors that can stimulate or inhibit cell proliferation, differentiation, inflammation or angiogenesis, depending on the state or need of the tissue. Fibroblasts divide relatively infrequently in resting tissues, but readily proliferate in response to tissue injury. This proliferation is generally driven by growth factors released from platelets upon wounding.

Fibroblasts in culture.

Fibroblasts were among the first cell type to be propagated *ex vivo*, largely because they grow so readily out of most tissue explants. Standard tissue culture conditions, particularly the

use of serum (which contains platelet factors), resemble a wounded environment and hence favor the growth of fibroblasts. It is therefore principally for historic reasons that fibroblasts, as opposed to other cell types, have been most extensively used to study cellular aging -- or, more accurately, cellular senescence -- in culture.

In the early 1960s, Leonard Hayflick and colleagues carefully documented the fact that normal human fibroblasts do not divide indefinitely in culture. Working with human fetal tissues, Hayflick showed that fibroblasts that grew out of the tissue explants initially proliferated well, with population doubling times of about 24 hours. He then serially passaged the cultures, allowing them to grow to confluence before dispersing and reseeding them at low density. With each passage, the cultures gradually accumulated non-dividing cells. After 50 or so doublings, the cultures consisted entirely of non-dividing fibroblasts. This phenomenon was termed the finite or limited replicative life span of cells. Hayflick was the first to connect this loss of proliferative potential with aging, and termed process that limits cell division cellular aging. It has also, and perhaps more accurately, been termed replicative senescence.

Since the 1960s, many cell types from many vertebrate species have been shown to have a limited replicative life span. Many of these studies have used cells in culture, but several have followed cells *in vivo* or cells serially transplanted into animals. Nonetheless, much of our knowledge about the causes and consequences of replicative senescence comes from studies of human fibroblasts in culture.

The senescent phenotype.

Fibroblasts undergo striking physiological changes upon replicative senescence. Three major features distinguish a senescent fibroblast from its presenescent (early passage)

counterpart. These are an irreversible arrest of cell cycle progression, resistance to programmed cell death (apoptosis), and a shift in function such that the cells no longer maintain the stromal extracellular matrix. Together, these changes are termed the senescent phenotype.

Growth arrest. Senescent cells, whether fibroblasts or other cell types, irreversibly arrest growth with G1 DNA content. This growth arrest is due to two types of changes in gene expression: repression of certain genes that are essential for cell cycle progression, and overexpression of certain genes that inhibit the cell cycle. In the case of fibroblasts, examples of genes that are repressed include those encoding the FOS proto-oncoprotein and the E2F1 transcription factor. FOS is important for the ability of fibroblasts to progress from quiescence (G0) through the G1 phase of the cell cycle, whereas E2F1 is important for entry into the S phase. Among the growth inhibitory genes that are overexpressed in senescent fibroblasts are those encoding the p21 and p16 proteins. p21 and p16 inhibit cyclin-dependent protein kinases, the activities of which are essential for G1 progression. p16 is a tumor suppressor gene and appears to be critical for maintaining the senescence growth arrest in many if not all cell types.

Resistance to apoptosis. Fibroblasts, and several other cell types (for example, T lymphocytes and epidermal keratinocytes) are more resistant to certain, although not all, stimuli that normally induce apoptosis. Consequently, senescent cells are quite stable. They have been shown to persist for many months or longer in culture. In addition, as discussed below, they very likely also persist *in vivo* and hence accumulate with age.

Altered morphology and functions. One of the most visible changes that occur when cells reach replicative senescence is a change in morphology. Cell size or volume increases,

often reaching double or more the presenescent size, and the cells accumulate intracellular vesicles, many of which are lysosomes. Fibroblasts become flatter and more irregularly shaped, showing more prominent intracellular actin fibers. For most cell types, the senescent morphology is quite distinct from that of proliferating, quiescent or terminally differentiated cells from the same lineage.

The striking change in cell morphology very likely reflects, at least in part, the functional changes that accompany replicative senescence. In the case of fibroblasts, upon senescence the cells switch from a matrix producing phenotype to a matrix degrading phenotype. Senescent fibroblasts produce less collagen and elastin, which form the major structural and elastic fibers of the stroma, than presenescent cells. The decline in collagen and elastin production is not due to a general decline in matrix production because fibronectin, a cell-associated matrix molecule, increases. In conjunction with the decline in collagen and elastin, senescent cells overexpress several matrix metalloproteinases. These enzymes degrade the stromal fibers and extracellular matrix. Senescent fibroblasts also secrete pro-inflammatory cytokines and a variety of epithelial cell growth factors. Thus, senescent fibroblasts, despite their inability to divide, adopt a phenotype that at least partially resembles a wounding response. As discussed below, senescence-associated functional changes may have a greater impact on the aging organism than the loss of cell division potential.

Causes of the senescence response.

The senescent phenotype was first and most thoroughly described for fibroblasts that reached replicative senescence. During the 1990s, several lines of evidence showed that cells sense the number of divisions they have completed by the length of their telomeres. Telomeres

shorten with each round of DNA replication owing to the biochemistry of DNA synthesis, and the fact that most normal somatic cells, including fibroblasts, do not express the enzyme telomerase. Fibroblasts, and many other cell types, arrest growth with the senescent phenotype when they acquire one or more critically short telomere.

During the late 1990s, it became apparent that a number of stimuli other that short telomeres also induce fibroblasts and other cells to arrest growth with a senescent phenotype. These stimuli include certain levels and types of DNA damage and the expression of certain oncogenes. In addition, conditions or agents that decondense chromatin, which causes silenced genes to be expressed, induce a senescence response in normal human fibroblasts. These stimuli induce a senescence response independent of cell division and telomere shortening, and the process that leads to the senescent phenotype has thus been termed cellular senescence. Thus, replicative senescence is a special example of the more general process of cellular senescence. Short telomeres, DNA damage, oncogene expression and loss of gene silencing have all been implicated in the initiation or promotion of cancer. For this and several other reasons, cellular senescence is thought to be important for suppressing tumorigenesis.

Cellular senescence *in vivo*.

Several lines of evidence suggest that senescent cells, including fibroblasts, exist and accumulate with age in at least some mammalian tissues *in vivo*. First, progressive telomere shortening, a hallmark of replicatively senescent human cells, occurs in some human tissues and cells (for example, segments of the aorta and T lymphocytes) with increasing age. Second, some -- but not all -- studies show that fibroblasts from older donors have shorter replicative life spans than fibroblasts from younger donors. The discrepancy among studies may be due to genetic

variation among individual donors or, more likely, the variation within even a single tissue in replicative history of the small regions that are sampled in biopsies. Indeed, there is substantial scatter in the data from even in the most convincing studies showing an inverse correlation between donor age and replicative life span of fibroblasts from skin biopsies. Third, in the mid-1990s, a marker enzyme was described that increased when cells were induced to senesce by a variety of stimuli. Cells expressing this marker increased with age in some human and primate tissues. This marker enzyme, termed the senescence-associated beta-galactosidase (SA-Bgal), is expressed by replicatively senescent fibroblasts, epithelial and endothelial cells, and other cell types in culture. It is also expressed by fibroblasts induced to undergo a senescence arrest in response to DNA damage, oncogene expression and agents that decondense chromatin. Fibroblasts and keratinocytes expressing SA-Bgal increase with age in human and monkey skin biopsies. In addition, retinal pigmented epithelial cells expressing this marker increase with age in human retina. Together, these findings suggest that senescent cells, whether induced by cell division or other stimuli, progressively accumulate in at least some mammalian tissues.

Role of fibroblasts in aging stroma.

The role of fibroblasts in aging tissue has been most extensively studied in mammalian skin. The skin is composed of two primary layers: the epidermis, which contains the major epithelial cells of the skin (epidermal keratinocytes) and the dermis, which is the stromal layer of the skin. As with other stroma, the dermis is maintained in large part by fibroblasts, which secrete dermal collagens, elastin and other extracellular matrix components. When the skin is wounded, fibroblasts secrete proteases to degrade the wounded matrix, and then synthesize new matrix. The fibroblasts also secrete growth factors to stimulate the keratinocytes to proliferate and close the wound, and cytokines to attract macrophage to engulf and degrade debris.

Young skin is characterized by thick epidermal and dermal layers, and relatively efficient wound healing. The epidermis contains numerous invaginations, or rete ridges, and the dermis contains dense collagen. Old skin, by contrast, is characterized by a thinner epidermis that contains fewer and shallower rete ridges. The dermis also becomes thinner, showing a marked loss of collagen and other fibers. Changes in the dermis are in large measure responsible for the loss of elasticity and wrinkling that is the hallmark of aging skin. In addition, wound healing slows with age. Aging is particularly sensitive to environmental influence in the skin. Sun (ultra-violet light)-exposed skin ages much more rapidly than sun-protected skin.

As discussed above, senescent fibroblasts appear to increase with age in human dermis, and senescent fibroblasts constitutively secrete factors that, ordinarily, are secreted only transiently during wound healing. These factors include interstitial collagenase and elastase, matrix metalloproteinases that degrade dermal collagens and elastin. Ultra-violet light can also induce these metalloproteinases, as well as cellular senescence, in fibroblasts. Thus, some of the hallmarks of aging skin – loss of dermal elasticity and wrinkling – is likely due at least in part to the secretion of metalloproteinases by fibroblasts, which, in turn, may be due to cellular senescence and/or environmental exposure to ultra-violet light. Senescent and ultra-violet damaged fibroblasts also secrete enzymes that degrade the basement membrane, the dense matrix onto which the epithelial cells are organized. This may contribute to the age-dependent thinning of the epidermis and loss of rete ridges, because the basement membrane is critically important for the proper organization and function of epithelial cells.

Age-dependent changes in fibroblast physiology may also contribute to the increased incidence of cancer that is a hallmark of mammalian aging. Several lines of evidence suggest mutations and loss of normal tissue structure very likely synergize to generate the exponential

rise in cancer that occurs with age. Tissue structure and integrity are critically dependent on an intact stromal and basement membrane, both of which are disrupted by senescent or damaged fibroblasts. In addition, senescent fibroblasts secrete epithelial growth factors, which can stimulate the growth of epithelial cells that have acquired potentially oncogenic mutations.

In summary, fibroblasts undergo physiological changes with age. These changes are induced by environmental and intrinsic factors, and disrupt the integrity of the stroma and basement membrane. Both these structures are critical in order for epithelial cells, and hence tissues, to carry out their normal functions. These structures are also important for suppressing the progression of cancer.

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